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Comparison of Tumor Response in Nude Mice and in Patients*

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Abstract

Four hundred and six human tumors of different origin were transplanted subcutaneously into nude mice. Histological evidence of viable tumor cells was obtained in 79%, rapid tumor growth after 3 months in 51%, and 49% of these tumors were transferred in serial passages. Most of the latter tumors were suitable for drug testing. In order to investigate whether tumor response in nude mice is consistent with patient response, 80 comparisons were performed comprising 55 tumors. Comparisons included carcinomas of the large bowel (27), lung (13), stomach (9), melanomas (8), breast (4) and 19 other tumors. Combination chemotherapy was more successful than single agent therapy (37 vs 14% remission rate). In 21 patients tumors went into remission, consistent with 19 cases in the nude mouse system. In 59 cases the patient did not respond to therapy, corresponding to 57 cases in the nude mouse system. Xenografts enabled correct predictions for resistance in 96% and for tumor response in 90% of cases. Despite great efforts to obtain a large number of comparisons, only 32 test results were available before the patients needed treatment. Therefore, our study has shown that the xenograft system will not have practical significance in determining individual patient treatment. Limitations are the duration of testing, the take rate of only 50% and the charges for nude mice. However, the highly correct prediction rates for tumor sensitivity and resistance validates human tumor xenografts as tumor models for testing new drugs and combinations. Furthermore, xenografts can be used as tumor source for experiments in cell culture.

Introduction

The response rates of the different human cancers to single agent and combination chemotherapy are well-known today. However, we cannot predict whether an individual patient will benefit from treatment. It might be possible to individualize drug regimens by testing a patient's tumor to a wide range of chemotherapeutic agents prior to treatment administration. For this purpose, several *in-vitro* "predictive assays" have been employed. They include long-term culture and evaluation of morphological changes (5), biochemical assays with inhibition of DNA or RNA synthesis (9), the *in-vitro* colony formation (6, 10), measurements of cell viability with dye exclusion assays (11) as well as reduction of tumor cells as measured by cytofluorometry (8). These *in-vitro* systems yielded highly correct prediction rates for tumor resistance. However, no system could obtain clinical significance in selecting drugs for individual patient treatment.

Over the past 10 years, most of the solid human tumors have successfully been grown in athymic nude mice. For chemotherapy experiments this *in-vivo* system presents advantages in comparison to *in-vitro* drug exposure. Drugs which must be activated or detoxified can be tested, and also drug combinations can be evaluated. During the past 6 years we have transplanted more than 400 different human tumors subcutaneously into nude mice. We will report on our take rates and the comparison of tumor response in nude mice and patients.

Methods

Animals

Athymic nude mice of the NMRI genetic background were used which were bred in our own nude mouse colony. The animals were kept in macrolon cages set in laminar flow rackets. They were

* Dedicated to G. W. Löhr, Freiburg, on the occasion of his 65th birthday

maintained as described by Fortmeyer (4). In the first passage tumors from men were implanted into male nude mice, tumors from women into female mice. All therapeutic experiments with the exception of testicular cancers were carried out in female mice.

Tumors

Over the past 8 years, 406 resected human malignancies were implanted subcutaneously into nude mice. Tumor slices of approximately $5 \times 5 \times 0.5$–1 mm diameter were implanted in the flanks of the animals, in the first passage usually 16 fragments into 4 nude mice. When the tumors reached diameters of 1–1.5 cm, they were subpassaged, and the remaining tumor material was studied histologically. In therapeutic experiments 2 tumor fragments were implanted into each animal.

Tumor Growth Measurements

Tumor growth was followed weekly by measuring 2 perpendicular diameters. The product of the 2 diameters was taken as a measure for tumor size. Relative tumor size values were calculated for each single tumor according to tumor size on day X divided by tumor size on day 0 at the time of randomisation, multiplied by 100. The median tumor size was taken for evaluation.

Experimental Design of Testing

Testing was performed in serial passages usually between passage 2 and 6 when the tumor growth became more regular. Mice were selected randomly for the untreated or the test groups after 3–6 weeks when individual tumors presented the following properties: product of the 2 tumor diameters $\geq$ 10 mm^2, estimated depth of at least half of the smaller diameter. Tumors with a yellow color indicating a high amount of fibrous tissue were excluded. Using these criteria we never observed spontaneous regression or stationary growth behavior in the untreated control groups after 3–4 weeks.

At the time of randomisation the median product of the tumor diameters was 56 mm^2. Each test group consisted of 5–6 animals comprising between 6–10 evaluable tumors. At the time of randomisation several representative tumors were examined histologically. The experiment was completed when each single tumor had doubled its initial tumor size which was the case for the majority of the tumors after 4–10 weeks.

Table 1. Comparison of chemotherapy in nude mice and in the patient

Tumor type	No. of patients	Chemotherapy		
		Total	Combination	Single Drug
Colorectal	18	27	3	24
Lung, Small Cell	2	4	4	0
Non Small Cell	6	9	5	4
Stomach	6	9	6	3
Melanoma	7	8	7	1
Mammary	2	4	2	2
Sarcoma	3	4	4	0
Testicle	2	2	2	0
Bladder	1	2	1	1
Thymoma	1	2	2	0
Renal	1	2	1	1
Wilms Tumor	1	2	2	0
Miscellaneous	5	5	4	1
Total	55	80	43 (54%)	37 (46%)

Chemotherapy

Fifty-five patients were treated with established drug combinations (43 cases) or with single-agent chemotherapy (37 cases). After progression under the initial therapy a second-line therapy was administered in 25 cases. The tumor categories and the type of chemotherapy are shown in Table 1.

Patient follow up and evaluation of patient tumor response were performed as in clinical trials.

The treatment regimen in nude mice corresponded to clinical schedules, with the exception that treatment in mice was usually repeated after 2 weeks. Drugs, doses, schedules and route of administration are shown in Table 2. A dose around the LD10 after 14 days and around the LD20 after 28 days was considered to be the maximum tolerable dose in tumor-bearing immunodeficient nude mice. In immunocompetent non-tumor bearing mice 2 treatment courses with these dose schedules caused a lethality of less than 10% after 4 weeks. In combination chemotherapy drugs were administered at 15-minute intervals by different routes to avoid direct interactions of the drugs. In 2-drug combinations only 70–80% of the dose that would be used in single drug therapy could be given, and, accordingly, only 50–60% in 3-drug combinations.

Evaluation Parameters for Tumor Response

The tumors in nude mice were evaluated after maximum tumor regression, or after 3–4 weeks in non-regressing tumors. The effect of treatment was

Table 2. Maximum tolerable dose-schedules of anticancer drugs in tumor-bearing nude mice

Drug	Dose mg/kg/day	Schedule day	Route of administration	Day 14		Day 28	
				death/total	%	death/total	%
ACNU	20	1	i.p.	16/134	12	24/134	18
Adriamycin	8.0	1, 15	i.v.	22/267	8	58/260	22
CCNU	20	1	i.p.	33/197	17	50/217	23
Cisplatin	6.4	1, 15	s.c.	13/143	9	32/143	22
	8	1, 15	s.c.	19/ 98	19	30/ 98	31
Cyclophosphamide	200	1, 15	i.p.	18/180	10	41/180	23
DTIC	80	1–4, 15–18	i.p.	1/ 50	2	8/ 50	16
	300–350	1, 15	i.p.	5/ 53	9	11/ 53	21
Etoposid	24	1–3, 15–17	s.c.	17/123	14	23/123	19
Fluorouracil	40	1–4, 15–18	i.p.	28/232	12	45/232	19
	125	1, 8, 15	i.p.	4/ 32	13	7/ 32	22
Mitomycin-C	2.0	1, 15	i.v.	15/168	9	48/179	27
Vindesine	1.5	1, 8, 15	i.v.	15/299	5	33/294	11

ACNU = Nimustine
CCNU = 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea
DTIC = Dacarbazine

classified in the xenograft system and in the patients as remission (product of 2 diameters < 50% of initial value), minimal regression (51–75%), no change (76–124%) and progression (≥ 125% of initial value). All patients had measurable lesions. Evaluation was usually performed after 2 treatment cycles or after maximal regression. The evaluation of tumor response in nude mice and in the patients was performed by different physicians.

Results

Take Rates and Growth Behavior

The take rates and the growth behavior of 406 different human malignancies are shown in Table 3. Histological evidence of viable tumor tissue was observed in 321 tumors (79%). Rapid tumor growth, defined as a tumor size of at least 60 mm² (a × b) after 90 days, was observed in 209 tumors (51%), and slow growth in 112 tumors (28%). Most of the rapidly growing tumors and in rare cases also initially slowly growing tumors were transferred to serial passages.

One hundred and ninety-seven malignant tumors (49%) were subpassaged at least 3 times. These high take rates were initially found in colorectal cancers only. After refining the technique, e.g. by selecting viable tumor tissue, similar take rates were observed in most of the other malignancies as well, with the exception of mammary cancers. One hundred and eighty regularly growing tumors of different tumor categories were selected as tumor models. Most of them were frozen in liquid nitrogen and are available for therapeutic and biological studies (1, 2).

Table 3. Transplantation of human tumors in nude mice. Take rate and growth behavior

Tumor type	Total number	Take[a]	Rapid[b] growth	Serial[c] passage
Colorectal	94	73 78%	49 52%	53 56%
Stomach	46	32 70%	13 28%	14 30%
Lung -				
Non Small Cell	70	62 89%	47 67%	42 60%
Small Cell	13	11 85%	7 54%	8 62%
Sarcoma	33	30 91%	22 67%	18 55%
Melanoma	33	29 88%	19 58%	18 55%
Hypernephroma	30	20 67%	13 43%	10 33%
Miscellaneous	87	64 74%	39 45%	36 41%
Total	406	321 79%	209 51%	197 49%

[a] histologic evidence.
[b] tumor surface (a × b) ≥ 60 mm² after 90 days.
[c] at least 3 passages.

Comparison of Tumor Response

Fifty-five tumors allowed 80 comparisons between tumor response in nude mice and in patients. In colorectal cancers 24 comparisons were evaluated using single-agent chemotherapy with 5-fluorouracil or nitrosourea and 3 employing combination chemotherapy. Most of the other tumors were treated with combination chemotherapy (40 times);

Table 4. Comparison of tumor response to chemotherapy in nude mice and in the patient

Mouse/Patient	Total
Remission/Remission	19
No Remission/Remission	2
No Remission/No Remission	57
Remission/No remission	2

Table 5. Comparison of tumor response in nude mice and in the patient according to tumor category

Mouse/ Patient	Total	Colo-rectal	Stom-ach	Lung / \ NSCLC	SCLC	Mela-nomas	Breast	Sar-coma	Other[a]
R /R	19	2	4		4		2		7
NR /NR	57	23	5	8		7	2	4	8
NR /R	2	2							
R /NR	2			1		1			
Total	80	27	9	9	4	8	4	4	15

[a] 2 testicle, 2 bladder, 2 thymoma, 2 renal, 2 epidermoid of skin, 1 thyroid cancer, 2 Wilms tumor, 1 penile cancer, 1 ovarian cancer.

only 13 with single agent chemotherapy. Mammary cancers were initially treated with tamoxifen and upon progression with the combination of cyclophosphamide, methotrexate and fluorouracil. Stomach cancers were treated with the FAM combination (fluorouracil + adriamycin + mitomycin-C), and small-cell lung cancers with a combination of adriamycin, vincristine and cyclophosphamide, upon progression with cisplatin and VP-16.

The overall results are given in Table 4. A total of 21 patients went into remission, corresponding to 19 cases in the nude mouse. Fifty-nine patients did not respond to treatment, which was consistent with 57 cases of therapy resistance in the nude mouse system. Overall, xenografts gave correct predictions for resistance in 96% and for tumor response in 90%.

Combination chemotherapy was more successful than single-agent chemotherapy. Out of the 43 combinations given, 16 (37%) effected a remission in the patients, as compared to 5 out of 35 (14%) of single-agent chemotherapies. Single-agent therapy was successful in 4 patients with colorectal cancer and in 1 stomach cancer case. Tumor response according to tumor type is shown in Table 5. The growth curves of 2 xenograft tumors are shown in Figures 1 and 2. Details of comparative chemotherapy in individual tumors have been described recently for 55 comparisons (3).

Potential of the Nude Mouse System as Predicitive Assay

During a period of 5 years great efforts were made to obtain a large number of treatment comparisons. Approximately two thirds of the tumors were obtained during surgery of the primary tumor with no immediate need to treat the patient with chemotherapy. We tried to have the testing result in the nude mouse available before the patient needed treatment. Testing was performed between passage 2 and 6 when the tumor growth became more regular, so that the results became available at the earliest 4 months after surgery in rapidly growing tumors and 2 years after surgery in some cases of very slowly growing tumors.

In 28 cases the test result was available in the xenograft system before the patient needed chemotherapy, in 18 cases the tumor was treated simultaneously in the nude mouse and in the patient, and in 34 cases the patients were treated first (Table 6).

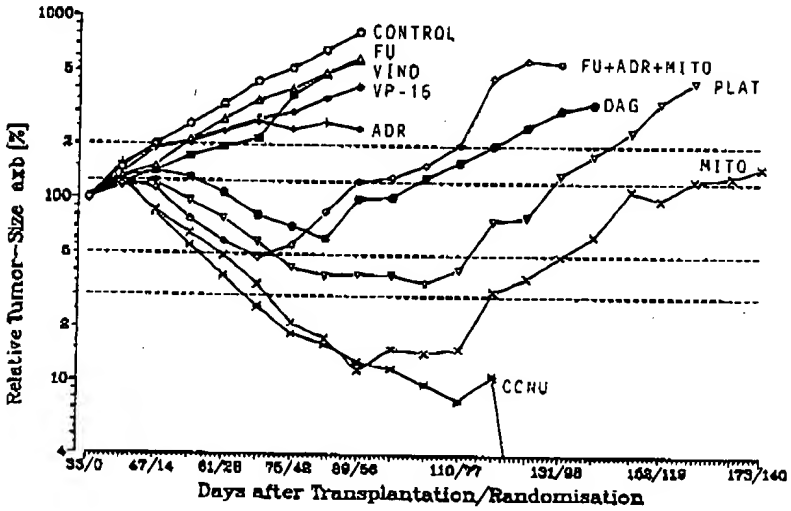

Fig. 1. Effect of single agent or combination chemotherapy on the growth of stomach cancer xenograft GXF 281. ADR = adriamycin; DAG = dianhydrogalactitol; FU = 5-fluorouracil; MITO = mitomycin-C; PLAT = cisplatin; VIND = vindesine; VP-16 = etoposid

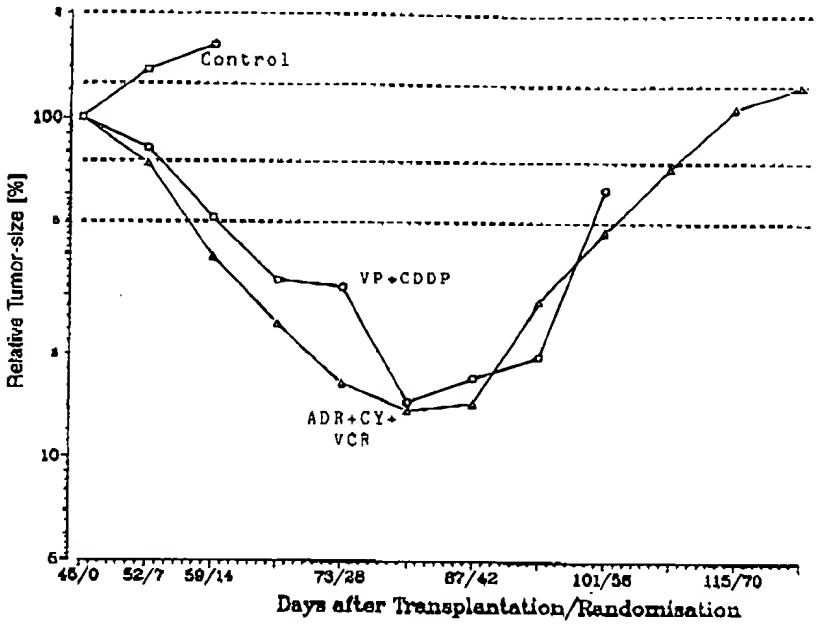

Fig. 2. Effect of single agent or combination chemotherapy on the growth of small-cell lung cancer xenograft LXF 177. □ = control; ○ = etoposid (VP) 24 mg/kg/day s.c. days 1–3, 15–17 and cisplatin (CDDP) 3.2 mg/kg/day s.c. days 1–2, 15–16; △ = adriamycin (ADR) 4 mg/kg/day i.v. days 1,15 and cyclophosphamide (CY) 150 mg/kg/day s.c. days 1,15 and vincristine (VCR) 0.5 mg/kg/day i.p. days 1,15

Table 6. Comparison of tumor response in nude mice and in the patient

	No.
Prospective[a]	28
Simultaneous	18
Retrospective[b]	34

[a] test result from nude mice available before patient needed chemotherapy.
[b] patient treated first.

Therefore, we can conclude that the xenograft system will not have practical significance in determining patient treatment.

Discussion

The main purpose of this study was to evaluate the significance of the xenograft system as a predictive assay for the individual treatment of patients and, furthermore, to validate the potential of the human tumor-nude mouse system for testing new drugs. Advantages include the highly correct prediction rates for resistance and sensitivity of a tumor. The testing is reproducible and also combinations and drugs which must be activated or detoxified can be studied. The highly correct prediction rates for sensitivity in comparison to *in-vitro* systems must be emphasized. In the stem cell assay and in biochemical assays only one half to two thirds of the drugs that were predicted to be effective were active in the clinic (6, 9). A better prediction for effective drugs was reported by Shorthouse et al. (7) with xenotransplantation into immunosuppressed mice.

However, our experience has shown that the nude mouse system will not have practical significance for predicting patient treatment. A major limitative factor is the duration of testing, requiring serial passage and thus a time period of at least 4 months in rapidly growing tumors, up to 2 years in slowly growing tumors, before test results can be obtained. In addition, nude mice are expensive; they need special conditions behind laminar flow barriers to avoid infections. Another limiting factor is the testing rate, since only half of the implanted tumors can be transferred to serial passages. Whereas the xenograft system cannot be used as a clinical routine method, the highly correct prediction rates for tumor sensitivity and resistance validate human tumor xenografts as human models for testing new drugs.

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